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ORIGINAL PAPER

Longevity of *Juniperus procera* seed lots under different storage conditions: implications for ex situ conservation in seed banks

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Abstract: *Juniperus procera* Endl. is economically important timber species, but its populations are extremely small and fragmented in its natural habitat, thus, calling for immediate ex situ conservation. Here we examined the effects of seed sources and storage temperature on the longevity of *Juniperus procera* seed lots through collection and preservation of seeds in seed banks. Seeds were collected from nine sites across the species natural distribution in Ethiopia and stored in four warehouses: modern cold room (5°C), mud house (15°C), concrete block house (17°C) or corrugated iron house (20°C) for 42 months. Every three months, a random sample of stored seeds were drawn and tested for germination. A highly significant variation ($p < 0.01$) in germination of stored seeds was observed among different storage environments, seed lots, and duration of storage. Over the storage period, seeds stored in the cold room had the highest mean percentage germination, followed by the mud house, corrugated house and blocket house. The cold room (41%) and the mud house (38%) maintained the same level of germination as the intitial germination of the seedlots (42%). The variation in longevity of stored seeds was significnatly correlated with the initial germination of seed lots ($r > 0.80$; $p < 0.01$). Cold storage also resulted in enhancement of germination through its stratification effect that terminated the non-deep physiological dormancy of juniper seeds. In conclusion, seed lots with good initial germination can be effectively stored in cold room (5°C) up to four years. In the absence of modern cold stores, mud houses can be used as a good alternative to store seeds at local level.

Keywords: germination; African pencil cedar; Ethiopia; seed zones; seed storage

Introduction

Juniperus procera Endl. (Cupressaceae), commercially known as African pencil cedar, is an evergreen dioecious tree that grows up to a height of 40 m and a diameter at breast height of 3 m (Pohjonen and Pukkala 1992). Its natural distribution extends from Yemen and southwestern Saudi Arabia to east and southern Africa, with altitudinal range of 1 100– 3 500 m and annual rainfall range of 300–1 200 mm (Friis 1992). It produces timber of high economic importance that can be used for manufacturing of lead-pencil, construction and lining of buildings, as well as for a variety of outdoor works owing to its fine texture, straight grain, medium hardness, resistance to termite attack and workability (Bekele et al. 1993). Mixed Juniper forests once occupied large mountain forests in the north, north-west, central and southeast highlands of Ethiopia (Friis 1992). Today, Juniper populations are extremely small and fragmented in its natural habitat due to anthropogenic disturbance (mainly logging). It is, thus, considered as endangered species that should be given the highest priority for conservation (WCMC 1998).

There is an urgent need for ex situ conservation of this species through collection and preservation of seeds in seed banks while simultaneous establishing new plantations in its natural habitat. Seed banking has regained a wider recognition as a relatively new and under-exploited tool in combating the loss of global biodiversity (Hong et al. 1997; van Slageren 2003; Scande et al. 2004). The Millennium Seed Bank Project, conceived on the basis of Convention on Biological Diversity, agreed upon at the United Nations' Earth Summit in 1992, is initiated to address the much-neglected need for conservation of wild species in the (semi-) arid regions of the world, with the principal aim of safe-

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guarding 10% of the world's flora against extinction (van Slageren 2003). Philip et al. (2010) further stressed the importance of ex situ conservation in seed banks under climate uncertainty as invaluable tool for conserving the maximum amount of genetic diversity in the minimum space for possible re-introduction and habitat restoration program in the future. Furthermore, many species produce seeds at long interval, from a few years to many years; therefore, storage is a bridge that allows securing the supply of required quantity of viable seeds at the time of sowing. In this case, a seed bank serves as a buffer between demand and production. The global effort to conserve biodiversity in seed banks should be supported by national and local initiatives so as to boost the success of biodiversity conservation at large. Thus, an understanding of factors influencing the longevity of seeds, the length of time seeds can stay viable during storage, is of paramount importance to effectively conserve and sustainably use genetic resources.

The storability of a seed lot is primarily determined by the vigor of the seed at maturity and level of deterioration at the time it enters storage (Albrecht 1993; Schmidt 2000; Demelash et al. 2004). A reduction in storage potential is one of the specific consequences of seed deterioration and is highly influenced by its pre-storage history and the storage conditions (Roberts 1973; Smith and Berjak 1995; Marcos-Filho and McDonald 1998). In addition, seed longevity depends on the seeds themselves and the condition around them (Murdoch and Ellis 2000). Based on seed storage behavior, three categories have been identified: orthodox, recalcitrant and intermediate. Orthodox seeds can be dried to low moisture contents (up to 5%) and can be stored over a wide range of environments without losing their viability (Roberts 1973). Long-term storage of orthodox seeds is possible in cool, dry environments. In contrast, recalcitrant seeds cannot withstand drying and loss their viability rapidly with further drying (Roberts 1973). Consequently they can only be stored at high moisture contents close to fully imbibed condition for short periods at moderate temperatures, above zero temperature (Roberts 1973; Sivakumar et al. 2006a, b). The intermediate category exhibits a behavior in between orthodox and recalcitrant seeds (Ellis et al. 1990), i.e., such seeds can be dried up to 7%–10% moisture contents depending on the species, but further drying may result in more rapid loss in viability of stored seeds (Ellis et al. 1990). In addition, the dry intermediate seeds of tropical species may loss their viability rapidly with reduction in storage temperature below a certain level (Ellis et al. 1990, 1991a, b; Hong and Ellis 1992).

Thus, longevity under storage can be extended by minimizing the limiting environmental factors, such as storage temperature, moisture content and oxygen partial pressure (Roberts, 1972), as well as enhancing initial seed lot quality that affect the viability of stored seeds. Over the years, appropriate conditions for long-term storage have been developed; i.e., storage facilities with controlled temperature and humidity conditions. For seeds that cannot be stored in such facilities, cryopreservation technology, defined as the storage of viable cells, tissues, organs and organisms at ultra low temperatures, has been developed, where tissues/organs are usually stored in liquid nitrogen (LN) and/or its vapor phase, at temperatures of ca. −196°C to −140°C (Benson 2008). Despite immense advances in seed storage research, the only available information about the storability of *J. procera* seeds is that of Teketay and Granström (1997), demonstrating lack of significant decline in germination during dry storage (orthodox behavior). Previously, we also reported a significant variation in germination among seed sources (Mamo et al. 2006), but whether this variation influences the storability of seeds is not known. Hence, this study was performed to examine the effects of different seed storage temperatures and seed sources on the longevity of *J. procera* seeds during storage for 42 months.

Materials and methods

Seed collection and processing

Cones of *J. procera* were collected from nine populations, representing the natural distribution of the species in Ethiopia. Populations were selected following the tree seed zone system developed for the country (Aalbaek 1993). The geographic location and climate conditions of the populations from which seeds were collected for the present study are shown in Table (1) and Fig. (1). Yabelo and Shakiso populations represent the southern range of distribution of the species. Wof-Washa, Chilimo and Kolobo represent the central distribution and Dodola, Kofele, Diksis and Hirna represent the widest range of distribution of the species in the southeastern highlands (Friis 1992).

Table 1. Geographic locations and climatic conditions of seed collection sites (Source: Aalbaek, 1993 and own GPS reading)

Collection sites	Latitude (° N)	Longitude (° E)	Altitude (m)	Rainfall (mm)	Temperature (°C)
Yabelo	4.53	38.02	2040	744	18.9
Shakiso	5.43	38.58	1771	973	18.6
Dodola	6.55	39.17	2675	915	13.9
Kofele	7.10	38.41	2595	1228	12.5
Diksis	8.03	39.32	2764	1265	12.9
Kolobo	9.12	38.5	2500	1610	15.7
Hirna	9.16	41.11	2575	1200	13.9
Chilimo	9.25	38.25	2550	1610	15.7
Wof-Washa	9.45	39.45	2525	1047	14.4

Cones were collected from phenotypically superior trees. The number of sampled trees per site ranged from 7 at Wof-Washa to 19 at Kofele due to availabilty of reproductively matured individuals during seed collection. The height of the seed trees ranged from 10.6 to 19.7 m and the diameter at breast height varied beteween 23.9 and 88.6 cm. To ensure the maximum genetic variation, the selected seed trees were at least 100 m apart from each other (FAO 1995). The cones were collected from all accessible branches of each sample tree to avoid the effect of fruit position on seed germination. The bulk fruit collected from each site was transported to the seed laboratory at the Forestry Research Center of the Ethiopian Institute Agricultural Research within two to four days after collection

where seed processing, initial germination test and storage trials were carried out. The seeds were extracted by manual depulping, cleaned and sun-dried to 8% moisture content.

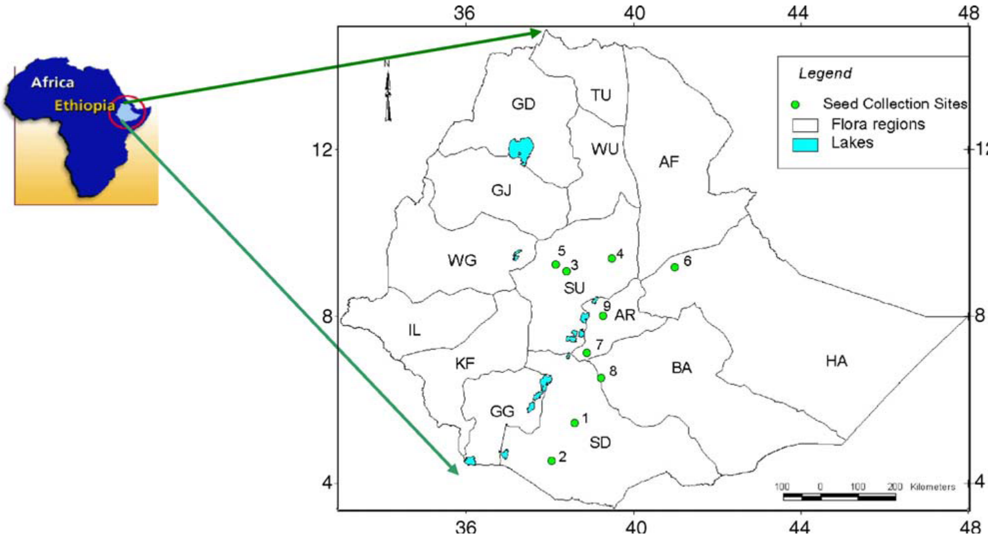

Fig. 1 Locations of seed collection sites

(1 = Shakiso, 2 = Yabelo, 3 = Kolobo, 4 = Wof-Washa, 5 = Chilimo, 6 = Hirna, 7 = Kofele, 8 = Dodola, and 9 = Diksis).

Storage trials

Prior to storage, the initial germination capacity of seeds of each population was tested. Subsequently, seed samples were sealed in paper bags and stored in cold room (5°C), mud house with pin roofed sheet (15°C), bricket house with thatched grass roof (17°C) and corrugated almunium house (20°C) for three and half years. Every three months, a random sample of 100 seeds from each population was taken for viability assessment. The viability of stored seeds was assessed by germination test, which was carried out under laboratory condition (room temperature) at the Forestry Research Center, Addis Ababa, Ethiopia. Four replicates of 25 seeds from each population were enclosed in 9.5 mm petri dishes on cotton that were continously moisten with distilled water. The petri dishes containing seeds were placed on a germination table in a completely randomised design. The germination process was monitored every day for 30 days, and germinated seeds were counted when the radicle reached 10 mm and had normal appearnce.

Data analysis

For each warehouse and seed lot, percentage germination was computed for each replicate as a proportion of number of seeds germinated after 30 days to that of sown seeds. The percentage germination was arcsin-transformed to meet the homogeneity of variance and normality assumptions for analysis of variance (Zar 1996). The analysis of variance was performed following the General Linear Model (GLM) for repeated measures:

$$Y_{ijk} = \mu + \beta_i + \lambda_j + (\beta\lambda)_{ij} + \varepsilon_{j(i)} + \varepsilon_{j(k)} \qquad (1)$$

where Y_{ijk} was the percentage germination, μ was the overall mean, β_i was the effect of the between-subject factors, *i* (warehouse, seed lots and their interaction), λ_j was the effect of the within-subject factor, *j,* storage time, $(\beta\lambda)_{ij}$ was the interaction of the between- and within subject factors. The parameters $\varepsilon_{j(i)}$ and $\varepsilon_{j(k)}$ are random errors of the between-subject and the within-subject factors, respectively with *k* number of replicates. Mauchly's test of Sphericity revealed that the homogeneity of variance assumption was not violated. Means that exhibited significant differences were compared using Tukey's test at 5% significant level.

To examine the magnitude of loss in viability due to storage, the change in germination of seed lots stored in different warehouses relative to their initial germination was computed for each storage time as follows:

$$\text{Relative change in germination (\%)} = \frac{G_t - G_0}{G_0} \times 100 \qquad (2)$$

where, G_t is the germination of stored seed lots at time *t,* and G_0 is the initial germination. Negative values indicate loss of germination during storage while positive values indicate enhancement of germination, as a result of dry storage.

Results

Initial seedlot characteristics

The initial characteristics of *J. procera* seedlots before storage are given in Table 2. The mean number of seeds per cone varied from 2 to 6, and seeds collected at Chilimo and Kolobo had the

largest mean number of seeds per cone (5) while those collected from Wofe-Washa and Hirna had the lowest mean nuber of seeds per cone (3). The mean seed mass ranged from 20.8 to 29.2 g/1000 seeds, and the highest being for seeds collected at Kolobo and the lowest for seeds collected at Wof-Washa. The purity of the seedlots was more than 91% and nearly similar in all seedlots. The initial mean percentage germination significantly varied among seedlots, ranging from 23% for Kofele and 64% for Wofe-Washsa.

Table 2. Initial characteristics of fruits and seeds of *J. procera* populations in Ethiopia. Germination percent followed by the same letters are not significantly different (α = 0.05).

Population	No. of seeds per cone	1000-seed mass (g)	Seedlot purity (%)	Germination (%)
Yabelo	3-5	25.3	96	44 [bcd]
Shakiso	3-5	22.2	98	57[ab]
Dodola	3-5	23.3	92	26[de]
Kofele	3-5	23.2	93	23[e]
Diksis	3-5	21.8	96	47[abc]
Kolobo	4-6	29.2	95	30[de]
Hirna	3-4	22.3	93	27[de]
Chilimo	4-6	27.4	97	55[ab]
Wofe-Washa	2-5	20.8	97	64[a]
Mean	3-5	23.9	95	42

Germination percent followed by the same letters are not significantly different (α = 0.05).

Longevity of stored seeds

Highly significant variations in germination of stored seeds were observed among different storage environments, seedlots, and duration of storage (Table 3). Over the storage period, seeds stored in the cold room had the highest mean percentage germination, followed by the mud house, corrugated house and blocket house (Fig. 2A). The cold room (41%) and the mud house (38%) maintained the same level of germination as the initial germination of the seedlots (42%). Among seedlots, the mean percentage germination of stored seeds was the highest for Shakiso and Wofe-Washa while the lowest value was recorded for Hirna, Dodola and Kolobo (Fig. 2B). For all seedlots and storage conditions, the mean percentage germination did not change much during the first 15 months of storage, but a sharp decline was noted from 21 months of storage up to 30 months, thereafter the change in mean percentage germination remianed constant (Fig. 2C).

There were significant first and second order interaction effects of storage environments, seedlots and duration of storage on percentage germination (Table 3). Relative to initial percentage germination, cold storage enhanced the germination of seeds collected from Chilimo by 7%–40%, from Shakiso by 21%–76%, from Wof-Washa by 12%–57%, from Diksis by 7%–38% through out the duration of storage, and from Yabelo by 5%–26% during the first 15 months of storage, while germination declined by 19%–52%, 2%–50%, 24%–64% and 26%–67% for Kofele, Kolobo, Hirna and Dodola seedlots, respectively across the study period (Fig.3). For seeds stored in the mud house, the percentage germination was enhanced for Chilimo, Shakiso and Wof-Washa seedlots compared to other seedlots that lost the viability remarkably during extended storage. Storage in concrete block house enhanced the percentage germination of seeds collected from Chilimo (up to 18 months), Shakiso (up to 21 months), Wof-Washa (upto 21 months) and Yabelo (upto 9 months) relative to the initial percentage germination, while the percentage germination was dramatically reduced for Dodola (≤ 90%), Hirna (≤ 83%), Kolobo (≤ 83%), Kofele (≤ 95%) across the study period as well as for Diksis (≤ 86%) and Yabelo (≤ 98%) during extended storage. Seeds collected from Wof-Washa and stored in corrugated warehouse had better longevity and enhanced germination across the durations of storage, and Chilimo and Diksis seedlots showed good longevity up to 15 and 24 months, respectively. Storage in corrugated warehouse resulted in lost of viability for the rest of seedlots; particularly under extended storage.

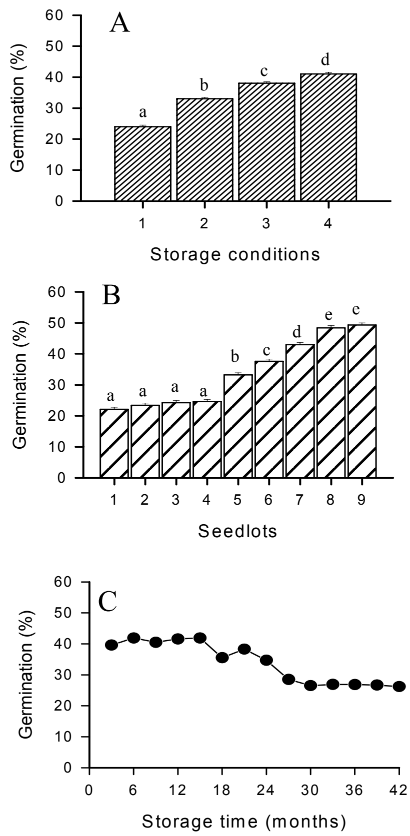

Fig. 2 Main effects of storage environments (A), seed lots (B) and storage time (C) on percentage germination of stored seeds of *J. procera* (Mean ± SE). Bars with different letter are significnatly different (p = 0.0001). In panel A, 1 = blocket house, 2 = corrugated house, 3 = mud house and 4 = cold room; in panel B, 1 = Kofele, 2 = Hirna; 3 = Dodola, 4 = Kolobo, 5 = Yabelo, 6 = Diksis, 7 = Chilimo, 8 = Wof-washa and 9 = Shakiso.

Table 3. Summary of repeated measures ANOVA for testing significant differences in percentage germination among storage conditions, seed sources and their interaction over 42 months of storage.

Source	d.f.	F	P
Between-subject factors			
Storage conditions (SC)	3	230.08	0.0001
Seed sources (SS)	8	245.49	0.0001
SC × SS	24	7.24	0.0001
Error	108		
Within-subject factors			
Year	13	67.39	0.0001
Year × SC	39	15.7	0.0001
Year × SS	104	2.56	0.0001
Year × SC × SS	312	1.96	0.0001
Error	1404		

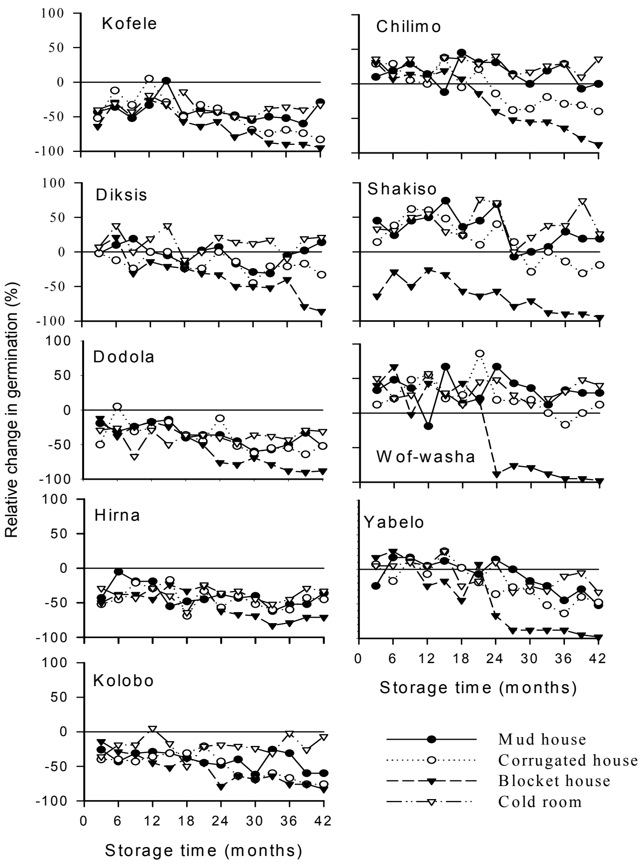

Fig. 3. Relative change in germination of *J. procera* seeds stored under different storage environments during the study period

Correlations between pre- and post-storage germination of seedlots

The correlations between intial germination and germination of seedlots stored for 42 months varied depending on the warehouse (Table 4). For seedlots stored in cold room, mud house and corrugated house, there was a significant positive correlation between pre- and post-storage germination. For seedlots stored in the concrete block house, the relationship was inverse, albeit not significant.

Table 4. Correlations between initial germination of *J. procera* seedlots and germination after 42 months of storage in different storage environments

Storage environments	Correlations	
	r	P-value
Cold room	0.878	0.002
Mud house	0.810	0.008
Corrugated house	0.850	0.004
Concrete block house	-0.306	0.423

Discussion

The initial germination of *J. procera* seed lots collected from different sites across its natural range of distribution varied significantly (Table 2). In most plant species, the degree of germinability of seeds greatly varies between and within populations (Mamo et al. 2006; Tigabu et al. 2007; Loha et al. 2006; 2008; 2009). Some of this variation can be of genetic origin, but much of it is known to be environmental, i.e. caused by the local conditions under which the seed matured. But the correlation between geo-climatic variables of seed collection sites and percentage germination was weak (Mamo et al. 2006), thus the variation in initial percentage germination could be related to maternal factors. Evidences suggest that maternal factors, such as position of the seed in the fruit/tree and the age of the mother plant during seed maturation markedly influence the germinability of seeds (Gutterman 2000). As seeds for the present study were collected from natural stands, it is legitimate to expect age variation among maternal parents.

This variation in initial germination was also reflected in the longevity of seed lots during storage, as evidenced from the strong positive correlation between pre- and post-storage germination of seed lots, depending on the storage condition (Table 4). The storability of a seed lot is primarily determined by the vigor of the seed at maturity and level of deterioration at the time it enters storage (Demelash et al. 2004). A reduction in storage potential is one of the specific consequences of seed deterioration, which in turn is governed by the genetic constitution, environmental factors during seed development and storage conditions (McDonald 1999). Seed deterioration occurs as a result of physiological and biochemical perturbations, such as impairment of energy synthesis mechanisms, reduction in respiration and biosynthesis activities, chromosomal aberration and DNA degradation and membrane alteration, which eventually reduces seed vigor, storability, germination capacity, and emergency potential (Smith and Berjak 1995; Marcos-Filho and McDonald 1998; Shen and Oden 2000; Demelash et al. 2004). In addition, the occurrence of Amadori and Maillard reactions causes protein inactivation and nucleic acid damage, leading to seed deterioration (Wettlaufer and Leopold 1991; Madhava and Kalpana 1994). The Amadori reaction involves a simple non-enzymatic glyca-

tion of reducing sugars on amino groups within proteins to form fructyosyl derivatives or glycated proteins while the Maillard reaction involves subsequent complex interactions between the glycated Amadori products to form complex brown-colored compounds.

In the present study, the warehouse made of corrugated iron and concrete blocks substantially reduced the longevity of stored seeds compared to the cold room (Fig. 2), which can be attributed to the relatively high temperature and diurnal fluctuation of temperature in these warehouses. The relatively high storage temperature might have accelerated seed deterioration by the action of microflora and/or heating effect that eventually had killed the seeds (Sivakumar et al. 2006b). This can be further corroborated by the inverse relationship between pre-storage germination and germination of seeds stored in concrete block house (Table 4), suggesting that temperature is the most important factor with a strong influence on seed longevity. We, however, noted enhancement of germination of stored seeds (Fig. 3), with marked variation between seed lots and storage environment (the enhancement being notably high in cold room). It has been suggested that *J. procera* seeds exhibit non-deep physiological dormancy (Teketay 1993) and stratification in moist sand at 3°C for 60 days is recommended to get good germination results (Albrecht 1993). Thus, the enhancement of germination during the entire storage period, particularly in cold room and mud house (Fig. 3), can be ascribed to the stratification or after-ripening effect, often recommended to overcome non-deep physiological dormancy (Baskin and Baskin 1998). For example, a positive effect of dry after-ripening was found in four out of 13 *Carex* species after three months of storage at 5°C, and in nine species after one month at 20°C (Grime et al. 1981). After-ripening or dry storage (10% moisture content) at ambient, 20°C or sub-zero temperature for a period of 30 weeks effectively breaks dormancy and results in high germination in *Strychnos nux-vomica* (Sivakumar et al. 2006b).

Conclusion

The current natural distribution of *J. procera* in Ethiopia is extremely small and fragmented, thus the species is considered as one of endangered species that should be given the highest priority for conservation. In this regard it is imperative to find appropriate conditions for ex situ conservation of the genetic bases on the long and short term bases. The findings from the present study showed that the longevity of *J. procera* seeds during storage is strongly dependent on seed sources and storage temperature. Seed lots with good initial germination and cold storage (5°C) effectively maintains the longevity of stored seeds, with added advantage in breaking the non-deep physiological dormancy. Storage in mud houses (15°C) also maintained the longevity of *J. procera* seed, next to the cold room. Thus, in the absence of modern cold stores or if not economically justifiable to establish one, mud houses can be used as a good alternative to temporarily store seeds at local level, assuming the established stores are free of pests and pathogens.

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